A COMPARATIVE STUDY OF THREE PHYTOPHTHORA DISEASES OF LILAC AND OF THEIR PATHOGENS

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With a diagram and plates 46 and 47

I. INTRODUCTION

A SERIOUS DISEASE of Lilacs caused by *Phytophthora Syringae* Kleb. has been recognized for many years in European lilac plantings. Very recently a second lilac disease attributed to *Phytophthora cactorum* (L. & C.) Schroet. has been found in America. A third lilac disease due to a distinct form of *Phytophthora* has been under observation at the Arnold Arboretum for several years. The present paper beside first reporting and describing the disease last named and its causal organism also reports a comparative study of the three Phytophthora diseases of Lilac with respect to the symptoms, etiology, and control of the diseases in question.

II. HISTORICAL

The first report of a Phytophthora disease of Lilac was that of Berkeley in 1881 (4) in which was described under the name *Ovularia Syringae* Berk. a fungus comparable to *Phytophthora infestans* (Mont.) de Bary which caused large brown patches on lilac leaves. Berkeley observed the production of conidia through the stomata and suggested that the conidia might germinate by means of zoospores. The following year Smith (31) first saw the oospores of Berkeley's fungus and described them. The germination of the conidia of Berkeley's fungus by means of zoospores was observed by A. S. Wilson in 1886 (33). Nine years later in 1905 (16) Klebahn published a short account of a disease of Lilac caused by what was in all probability the same fungus as that of Berkeley. The identity of Klebahn's fungus and that of Berkeley was not recognized, however, and Klebahn's disease was attributed to a new species and genus of fungus, *Phloeophthora Syringae* Kleb. According to Klebahn's observations the disease was seated in the cortex of mature lilac branches, was manifested by a browning and death of the cortex, and was apparently not related to any leaf disease. Klebahn succeeded in obtaining pure cultures of the fungus and in producing typical lesions on artificially inoculated plants. Although the sexual structures were seen, there was no apparent production of conidia, which latter fact, together with the location of the disease in the cortex of woody stems, led to the assumption of a new genus.

The following year Klebahn continued his studies (17) and brought out the relation of the disease to the abnormal environmental conditions of the lilac forcing industry. A much more extended account of the same disease is contained in a later work by the same author in 1909 (18), reviewed by Hasselbring in 1910 (13), in which he finally observed the conidia and recognized the true nature of the fungus. The name was accordingly changed to *Phytophthora Syringae* (Kleb.) Kleb. In this longer account Klebahn reported detailed investigations of the symptoms produced on stored and forced Lilacs, the morphology and biology of the fungus, the proof of the parasitism of *Phytophthora Syringae*, and the control measures which had been found to be effective in reducing the disease. In addition to infection experiments with Lilacs, the author also found the fungus to be capable of parasitism of a variety of other host plants. Klebahn's observations showed the disease to be present in 1909 in Hamburg and Cuxhaven, whence he believed it had been introduced from France. Later in 1909 Lustner (24) also reported it from Hohenheim and Frankfurt am Main. In 1910 Himmelbaur (15) repeated Klebahn's studies of *Phytophthora Syringae* and confirmed the latter in finding *P. Syringae* distinct from any previously reported species of *Phytophthora*.

By 1913 the fungus had spread to Holland according to the report of Schoevers (29) and was there likewise found to be causing a serious disease of cultivated lilac plants. The following year G. W. Wilson studied *P. Syringae* from the taxonomic standpoint (34), and beside confirming the earlier descriptions of the fungus the author was the first to point out the probable identity of Klebahn's fungus with that of Berkeley's. The name *Phytophthora Syringae* (Kleb.) Kleb., however, is retained. In 1918 Arnaud (1, 2) reported the appearance of the disease in France, where it was causing minor injury in a hedge of Lilac. Here for the first time the conidiophores of *Phytophthora Syringae* were observed occurring in nature on infected Lilacs.

Up to 1922 the disease had been reported from England, Germany France, and Holland. In that year Lafferty and Pethybridge (19) reported an isolation of *Phytophthora Syringae* from rotted apple fruit in Ireland. Specimens of lilac leaves probably injured by *P. Syringae* had also been received by the same authors. In the paper in question the authors reviewed the morphology of the sexual organs of *P. Syringae* and reported for the first time the presence of both amphigynous and paragynous antheridia within the species.

Phytophthora Syringae was shown to be capable of saprophytic life in the soil by de Bruyn in 1922 (5). Two years later Miss de

Bruyn published a continuation of her studies (6, 7) in which were reported an extensive series of infection experiments on Lilac. In the latter papers she found that the disease occurred in greatest destructiveness in those seasons in which there was abnormal rainfall in August or September. Infection was found to take place in the winter months, from December till April in the cortex, and from October till February in the buds. Hand picking of the leaves as a control measure was suggested, but in a later paper (8) the author found that hand picking of the leaves was so injurious to the blossoms as to eliminate it as a control measure.

The only record of *Phytophthora Syringae* in America is that of Hedges in 1929 (14). Miss Hedges found a fungus believed to be *P. Syringae* fruiting on several blighted young lilac shoots in Washington. The lesions had the appearance of those due to fire blight (*Bacterium Syringae*), and the attack was severe. Since the lesions described were on young shoots examined in May, it is possible that the *Phytophthora* found was one of the other two species here considered, since *P. Syringae* normally does not primarily attach the succulent tissues of lilac.

Phytophthora cactorum (L. & C.) Schroet., originally described as *Peronospora cactorum* by Lebert and Cohn in 1870 (20) and since investigated by many workers, is known to parasitize a great variety of host plants. On Lilac, however, it has been recognized only recently. In 1929 R. P. White described a disease of *Rhododendron* and Lilac from New Jersey, with which was associated this species of *Phytophthora* (35). Cross infection experiments proved that the same fungus was responsible for the disease in both hosts. On Lilac the disease takes the form of a dying-back of suckers and of leaf infections. Production of conidia was observed on the Lilac. As control measures for Lilacs, White suggested generous spacing of the plants, removal of dead wood, and use of a dormant spray of lime-sulphur together with summer applications of Bordeaux mixture.

With regard to the third type of *Phytophthora* causing disease in Lilac, no record has heretofore appeared in the literature.

In addition to the primarily pathological literature dealing with the lilac Phytophthoras, a number of purely mycological papers have dealt with *P. Syringae* and *P. cactorum*. An attempt will not be made to go into the taxonomy of these species at the present, but it may merely be said in passing that the two species are considered perfectly distinct by all of the leading students of the genus (G. W. Wilson, 1914, 34; Rosenbaum, 1917, 26; Leonian, 1925, 21; Tucker, 1931, 32).

III. MATERIALS AND METHODS

The cultures of *Phytophthora* employed in the present study were obtained from the following sources: (a) a culture of *Phytophthora Syringae* isolated by Miss de Bruyn from Lilac in Holland and obtained from the Centraalbureau voor Schimmelcultures at Baarn in 1928; (b) a culture of *Phytophthora cactorum* isolated from Lilac in New Jersey by R. P. White and sent to me by Dr. White in 1929; (c) several cultures of the same organism isolated from Lilac by the writer in 1929; (d) a third distinct strain isolated from Lilac in the Arnold Arboretum by the writer in 1929 and hereafter referred to as *Phytophthora* "Type A."

Stock cultures of the various strains were maintained on potato-dextrose agar. For the production of the spore forms special techniques were necessary. None of the strains produced sporangia in appreciable amount on potato-dextrose agar. For sporangium production the technique originally devised by Klebs was employed. Tiny fragments of mycelium of active cultures were transferred to large test-tubes each containing about 20 cc. of pea decoction. After several days growth in the pea broth at room temperature the mycelial mats were transferred to sterile pond water, the sterile water being renewed frequently. Distilled water did not prove satisfactory for this purpose. Abundant production of sporangia resulted at room temperature within 24 to 48 hours after transferral to sterile water. The production of sporangia was also induced by the conventional employment of Petri's mineral solution

(.4 gm. $Ca(NO_3)_2$ + .15 gm. KH_2PO_4 + .15 gm. $MgSO_4$
+ .06 gm. KCl + 1000 cc. H_2O),

although Petri's technique proved much less satisfactory than that of Klebs for the species of *Phytophthora* involved.

Oospore formation was brought about by the employment of special solid media, the requirements differing for the different strains of *Phytophthora* under consideration. *Phytophthora cactorum* readily reproduced sexually on a wide variety of solid substrata. Among these steamed corn-meal, steamed green bean pods, steamed carrot, lima bean agar, oatmeal agar, and corn-meal agar proved very favorable. *Phytophthora Syringae* produced oospores with apparent difficulty, sterile lilac leaf extract (10), steamed carrot, steamed corn-meal, and oatmeal agar in the order named yielding the most satisfactory results. *Phytophthora* "Type A" produced oospores abundantly on steamed green bean pods, lima bean agar, and corn-meal agar.

Since the morphology and the physiology of the genus *Phytoph-*

thora are subject to considerable variability according to the type of substrate employed, the reactions of the lilac Phytophthoras to various artificial media were studied in detail. The following media were used to this end:

Steamed corn-meal
Steamed green bean pods
Potato-dextrose agar
Lima bean agar
Oatmeal agar
} Prepared according to the formulas of Tucker (32).

Potato-dextrose agar (Filtrate of 200 gm. boiled potato + 25 gm. agar + 30 gm. bacto-dextrose + water to make 1000 cc.).

Malt agar (50 gm. malt extract + 25 gm. agar + water to make 1000 cc.).

Prune agar (50 gm. dried prunes + 25 gm. agar + water to make 1000 cc.).

Pea broth (Filtrate from 50 gm. dried split peas boiled until soft + water to make 1000 cc.

Steamed carrot cylinders.

In addition to the media indicated above, corn-meal agar with the addition of certain stains was employed in a brief study of the penetration of dyes *in vivo*.

The observations of the Phytophthora diseases in nature and the study of the physiology, morphology, and taxonomy of the fungi involved were supplemented by an investigation of the pathology of the various strains of *Phytophthora*. For this purpose several series of inoculation experiments were performed in which the strains of *Phytophthora* under consideration were inoculated into a variety of Oleaceous plants. Such inoculations were of various form, consisting of insertions of mycelium into injured leaf and stem tissues, placing of mycelium upon unbroken plant parts, and finally permitting suspensions of freshly liberated zoospores to come into contact with the plant tissues. The inoculated plants were retained in an Arnold Arboretum research greenhouse under conditions favorable for their growth, and the inoculations were protected in the conventional manner.

Finally, all of the available literature pertaining to the Phytophthora diseases of the Lilac was carefully assembled and compiled. The bibliography at the end of this paper accordingly contains a virtually complete list of the references to scientific studies dealing with one or another phase of the problem of Phytophthora disease in Lilacs.

IV. COMPARATIVE STUDY OF THE SPECIES OF PHYTOPHTHORA PARASITIZING LILAC

As a preliminary to a correct diagnosis of the Phytophthora diseases of Lilac and to a correct determination of the rôle played by the fungi involved in causing disease, a study of the comparative mycology of the fungi was essential. Such a study has been in progress and the experimental results and interpretations will be the subject for consideration in the present section. For convenience the experimental findings are grouped under the headings of physiology, morphology, and systematics.

A. PHYSIOLOGY

The genus *Phytophthora* has long offered to systematists a difficult problem. The morphological characters by which species of fungi are separated must necessarily be relatively invariable within the species in order that the specific differences may be determined with accuracy. Where clear cut morphological differences are lacking, as in the bacterial genera, the systematist is forced to turn to the more striking physiological characters as bases for specific distinction. The taxonomic studies of *Phytophthora*, first based upon what were believed to be sharp morphological criteria, have undergone a gradual evolution from purely morphological systems to those almost purely physiological. The reason for such an evolution in approach has been that many of the morphological characters formerly assumed to be constant within a species have since proved to be capable of wide variation according to the physiological environment. Thus the position of the antheridium relative to the oogonium was at one time felt to be a constant character in *Phytophthora* species. The work of Lafferty and Pethybridge (19) however has shown that in many species of *Phytophthora* both amphigynous and paragynous antheridia occur, although there is a tendency in a given species to form the great majority of the antheridia in one or the other manner. The size of the reproductive organs, again a character which is usually dependable in the fungi, is susceptible to such wide variation in *Phytophthora* that the only findings yielding results at all satisfactory are those based on extensive biometric studies. Other characters of the species such as method of conidial germination, method of zoospore germination, mycelial characters, and sexuality likewise exhibit high degrees of variability within a given species. Accordingly any mycological study such as the present one must necessarily be concerned both with physiological and morphological characters. Among the physiological criteria which may effectively be applied to *Phytophthora* are the rate and

type of growth upon various artificial media, the production of fructifications upon various media, and the relationship of temperature and pH to rate of growth. Accordingly these various physiological factors in relation to the lilac Phytophthoras will be discussed at this point.

RATE AND TYPE OF GROWTH ON VARIOUS MEDIA. During the three years in which the lilac Phytophthoras have been a subject of study in this laboratory they have been grown on a wide variety of nutrient substrata. Freshly-made media were always employed, made according to the formulae given in the preceding section. For the preparations of fresh vegetable products small wide-mouthed vaseline bottles were used as receptacles, while for the other media both culture tubes and Petri dish cultures were employed. All of the cultures indicated below were grown at a room temperature of 21° C. The results of these experiments are indicated in the table on pages 239–240. The terms used in description of the mycelium are those of Long and Harsch (23).

It is at once apparent from a consideration of the foregoing data that there is a distinct difference in behavior toward the various media of the three strains of *Phytophthora* at hand. On the whole *Phytophthora Syringae* is more divergent from the remaining two species than the latter are from each other. This greater divergence of *P. Syringae* is particularly evident in its weaker growth on all media. On the whole *Type A* resembles *P. cactorum* in the general features of its growth reactions. On the other hand, a number of differential reactions set it apart from *P. cactorum*. Thus one finds aërial mycelium in *Type A* on oatmeal, corn-meal, malt, and prune agars, while the mycelium of the other strains is wholly appressed on these media. The aërial mycelium of *Type A* is characteristically different from that of *P. cactorum*, being longer, relatively less branched, and frequently with a silky sheen. The three strains are perhaps most strikingly differentiated on steamed carrot slants. *P. Syringae* grows much more slowly than the other strains on this substrate at room temperature, its growth being very slight during the first week while the other two strains are able to occupy the entire slant in that time. Moreover *P. Syringae* is white with much aërial mycelium at the edges of the colonies. *P. cactorum* and *Type A* are both much more sodden. *Type A* is white except as the sodden appearance makes it difficult to determine the color, while *P. cactorum* is noticeably discolored due to myriads of oospores, its color being clay-color (Ridgeway). The powdery-sodden appearance of *P. cactorum* is also readily distinguished from the silky-sodden appearance of *Type A*. Thus a consideration of the manner and extent

TABLE 1. RATE AND TYPE OF VEGETATIVE GROWTH OF THE LILAC PHYTOPHTHORAS ON VARIOUS MEDIA (21° C.)

Substrate	Days after inoculation	Phytophthora Syringae	Phytophthora cactorum	Phytophthora "Type A"
Steamed corn-meal....	6	Mycelium extensive but thin, not forming a cottony mass. Colony more than 5 cm. in diameter.	As in *P. Syringae.*	As in *P. Syringae.*
	14	Mycelium as at 6 days.	As in *P. Syringae.*	As in *P. Syringae.*
Steamed green bean pods	6	Mycelium sodden and felty, not as extensive as in the following two strains. Colony about 3 cm. diameter.	Mycelium cottony to felty, forming a distinct pellicle over water. Colony about 5 cm. diameter.	Mycelium cottony, rather thinner than the last, forming a pellicle over water. Colony about 5 cm. diameter.
	14	Mycelium as at 6 days.	Mycelium as at 6 days.	Mycelium now more cottony than in the other strains.
Lima-bean agar.....	6	Mycelium appressed, none aërial whatever. Colonies only 2.5–4.0 cm. diameter.	Mycelium cottony to felty, compact, denser and shorter than in the next. Colonies 5.0–6.5 cm. in diameter.	Mycelium almost cobwebby, thin but long except at the margin of the colony where there is a 5 mm. zone which abruptly becomes appressed. Colonies 5–6 cm. diameter.
	14	As at 6 days.	As at 6 days.	As at 6 days. Hyphae long, thin, relatively unbranched.
Oatmeal agar.....	6	Mycelium completely appressed. Very thin and difficult to see. Colonies 2–4 cm. diameter.	Mycelium completely appressed but much more easily distinguished than the last because not so thin. Colonies 7–9 cm. diameter.	Considerable aërial mycelium present, cobwebby-downy but thin and distinguished with some difficulty. Colonies 7–9 cm. diameter.
	14	As at 6 days.	As at 6 days.	Much aërial mycelium present though none on the other two strains.

TABLE 1—Continued

Substrate	Days after inoculation	Phytophthora Syringae	Phytophthora cactorum	Phytophthora "Type A"
Corn-meal agar	6	Mycelium completely appressed, very thin and distinguished with difficulty. Colonies 2.5–3.5 cm. diameter.	Mycelium completely appressed, thin but extensive. Colonies 7–9 cm. diameter.	Mycelium much as in the last, thin, appressed, but becoming aerial (cobwebby) at the lower end of the slant. Aërial mycelium distinguished with difficulty. Colonies 7–9 cm. diameter.
	14	Mycelium as at 6 days. No aërial mycelium.	Mycelium as at 6 days. No aërial mycelium.	Aërial mycelium plainly visible.
Potato-dextrose agar	6	Mycelium sodden and slightly downy. Colonies 3 cm. diameter.	Mycelium subfelty, not at all sodden, rather compact, not long and silky as in the next. Colonies 5–6 cm. diameter.	Mycelium cottony with a silky sheen. Hyphae long. Colonies 6–8 cm. diameter.
	14	Growth moderate, sodden or slightly downy. Zonate in daylight.	Growth moderate, subfelty, azonate in daylight.	Growth strong, cottony, azonate in daylight.
Malt agar	5	Almost no growth.	Moderate, more or less sodden growth.	Extensive downy growth.
	14	Growth weak, appressed.	Growth moderate, appressed to downy.	Growth strong, appressed.
Prune agar	2	No growth.	Growth moderate, thin, appressed.	Growth strong, appressed to cobwebby.
	14	Growth very weak, appressed.	Growth moderate, appressed.	Growth strong, downy.
Steamed carrot	10	Growth very weak, downy.	Growth moderate, felty.	Growth strong, woolly.
	20	Growth weak. Downy, becoming sodden at the center.	Sodden but with such a covering of oospores as to give it a powdered appearance. Aërial mycelium downy where it appears (at center of colony only).	Sodden with none of the powdery appearance of the last. The scanty aërial mycelium is very silky.
Pea broth	3	Growth weak. Colonies .5 cm. diameter.	Growth strong. Colonies about 2.5 cm. diameter.	Growth strong. Colonies about 2.5 cm. diameter.

of growth upon various media offers evidence as to the existence of three distinct strains of *Phytophthora* parasitizing Lilac.

PRODUCTION OF FRUCTIFICATIONS ON VARIOUS MEDIA. A second type of physiological evidence is yielded by a study of the ability of *Phytophthora* to produce asexual and sexual reproductive organs on various media. Such a study has been carried on with reference to the lilac Phytophthoras and the results are summarized in Table 2 on page 242. The cultures were all made in the conventional manner, the normal pH of the cultures was not altered, and the cultures were all grown at 21° C except as otherwise indicated. In the section entitled "Klebs' technique" the fungi were grown for four or five days in sterile pea broth and then transferred to sterile pond water, the water being changed twice daily.

The data in Table 2 again reveal the striking difference between *P. Syringae* and the other two strains, although the latter two behave in a rather similar fashion with regard to the production of fructifications. Oogonia are apparently formed with some difficulty in *P. Syringae* and are absent on a number of media on which the other two fungi produce them in abundance. At the other extreme is *P. cactorum* which produces oogonia on many media in surprising numbers. For example it was seen in temperature experiments that *P. cactorum* will cover a Petri dish of corn-meal agar with a profusion of oospores in 4 days at 25° C. *Type A* behaves in an intermediate manner, forming numerous oospores on a variety of media but never to quite the same extent as *P. cactorum*. Except by the employment of Klebs' principle of suddenly removing the food supply, the production of sporangia is very limited in all the strains. None have ever been observed in artificial cultures of *P. Syringae* except as grown by the Klebs and Petri techniques. Frequently the sporangia formed in artificial culture are very irregular or abortive in form, being non-functional in the form of moniliform swellings on the hyphae, functional but bi-papillate, greatly elongated or asymmetric, etc. From the data presented one may conclude that with respect to the character of fructification on various media, *P. Syringae* is perfectly distinct from the other strains, while the latter, although resembling each other in the main do differ significantly in a number of features.

RATE OF GROWTH AT DIFFERENT TEMPERATURES ON THE SAME MEDIUM. A third physiological factor of value in differentiating species and of great importance in the economic considerations of Phytophthora diseases is temperature. Differential growth according to temperature has been seen to be of such value in species diagnosis that it ranks among the most useful characters on which

TABLE 2. PRODUCTION OF REPRODUCTIVE ORGANS BY THE LILAC PHYTOPHTHORAS ON VARIOUS MEDIA

Medium	Days after transfer	P. Syringae		P. cactorum		Phytophthora Type A	
		Sporangia	Oogonia	Sporangia	Oogonia	Sporangia	Oogonia
Steamed corn-meal	14	None	Frequent	None	Very abundant	None	Present, not abundant
Steamed bean-pods	14	None	None	Infrequent	Very abundant	None	Abundant
Potato-dextrose agar	14	None	None	Frequent	Fairly abundant	Very infrequent	Present, not abundant
Lima-bean agar	14	None	None	Numerous	Abundant	None	Abundant
Oatmeal agar	14	None	Present, not numerous	None	Numerous	None	Abundant
Corn-meal agar	14	None	Very infrequent	Not infrequent	Numerous	None	Numerous
Klebs' technique (21° C.)	1	None	None	None	Abundant	None	None
	2	Present	None	Numerous	Abundant	Numerous	None
	3	Present	None	Numerous	Abundant	Numerous	None
	4	Numerous	None	Numerous	Abundant	Numerous	None
Klebs' technique (8° C.)	1			None	None	None	None
	2			None	None	None	None
	3			Present	None	None	None
	4			Present	None	Present	None
Petri's mineral solution. From culture on:							
A. Corn-meal agar	5	Present, not abundant		Present, not abundant		Absent	
B. Potato-dex. agar	5	Absent		Infrequent		Infrequent	
C. Lima-bean agar	5	Very infrequent		Very infrequent		Infrequent	

recent Phytophthora keys are based, Hence it was thought desirable briefly to investigate the behavior of the Phytophthora strains under consideration at different temperatures. It proved practicable to work only with the temperatures above 21° C, but since *P. Syringae* is apparently the only strain of the three at hand which vegetates extensively at the cooler temperatures and since the temperature relations of *P. Syringae* have been studied in detail by previous workers, the data at hand are sufficient to indicate the pronounced differences between the three strains. The temperature study reported here was conducted with the fungi growing on homogeneous lots of corn-meal agar in constant temperature chambers with a temperature variation of no more than .5° C. Thirty plates of each fungus were used at each temperature. The results may be briefly summarized as follows.

At 29° C. none of the lilac strains of *Phytophthora* made appreciable growth. At 27° C. *P. Syringae* failed to grow as did most of the cultures of *Type A* and *P. cactorum*, although a few scattered cultures of each of the latter two strains showed a very limited growth. At 25° C. *P. Syringae* failed to grow, but *Type A* and *P. cactorum* grew very extensively, the colonies being from 75 to 90 mm. in diameter after 96 hours. Numerous oospores were present in the cultures of these latter strains after 96 hours. This temperature appears to be very near the optimum for both *Type A* and *P. cactorum*, as the growth is far more extensive at 25° C. than at room temperature. The upper limit of growth for *P. Syringae* is approximately 23° C., and the optimum, according to my own observations and to Tucker's more critical studies (32), is about 20° C. Thus a clear-cut distinction according to temperature requirements exists between *P. Syringae* and the other lilac strains of *Phytophthora*. *P. Syringae* grows best at 20° C., a temperature at which the growth of *Type A* and *P. cactorum* is only indifferent, while at 25° C., the optimum temperature for both *Type A* and *P. cactorum*, the growth of *P. Syringae* is wholly inhibited. Apparently the lilac strain of *P. cactorum* is somewhat less resistant to heat than the strains of *P. cactorum* studied by Tucker, all of which vegetated at least to 27.5° C. and some to 30° C. The practical bearing of temperature requirements of the lilac Phytophthoras will be considered later in relation to their pathology.

Relation of growth to hydrogen ion concentration. A fourth physiological characteristic useful in distinguishing Phytophthora species is the relation to hydrogen ion concentration. In order to investigate this matter an experiment was devised in which the fungi were permitted to vegetate in liquid media of varying hydro-

gen ion concentrations. Pea broth was selected as the medium to be used, since all three strains of *Phytophthora* from Lilac grow well in this decoction. A single large flask of pea broth was filtered and then divided into 16 equal portions. One portion was retained without altering the normal pH, which was found to be 6.5. The other portions were titrated with decinormal HCl and KOH to a series of pH values extending from 2.5 to 10.0 at .5 pH intervals. The pH determinations were made colorimetrically by the use of Clark's indicators. Each portion was now divided into three equal samples of 15–20 cc. each, the samples being placed in 6 x 1″ test tubes. The 45 tubes were then autoclaved and finally all the tubes of each complete series of pH values were inoculated with *P. Syringae*, *P. cactorum*, and *P. Type A* respectively. The fungi were permitted to develop for 5 days at room temperature at the end of which time the amounts of growth were compared. The results are given in the following table:

	pH OF MEDIUM:															
	2.5	*3.0*	*3.5*	*4.0*	*4.5*	*5.0*	*5.5*	*6.0*	*6.5*	*7.0*	*7.5*	*8.0*	*8.5*	*9.0*	*9.5*	*10.0*
P. Syringae	0	0	3	4	4	4	4	4	4	3	1	0	0	0	0	0
P. cactorum	0	2	3	4	4	4	4	4	4	4	4	4	4	2	2	2
P. Type A	0	2	3	4	4	4	4	4	4	3	3	3	3	2	2	0

(KEY: 0—No growth; 1—Very weak growth; 2—Weak growth; 3—Moderate growth; 4—Strong growth.)

The results of this experiment confirm the earlier findings in demonstrating *P. Syringae* to be markedly distinct from the other two lilac strains of *Phytophthora*. The latter two show virtually no difference, however, in their pH reactions. The extremely long pH range of all three of these strains, particularly the latter two, is worthy of note, and the high degree of toleration exhibited argues against the possibility that the pH reaction of host tissue might play a part in the immunity or susceptibility of a given host subject to these strains of Phytophthora.

In relation to the preceding experiment in hydrogen ion control, an experiment was also performed in which was investigated the ability of certain dyes to penetrate the living *Phytophthora* cell. The experiment took the following form. A litre of clear corn-meal agar was prepared in the customary fashion. When liquid this was divided into a number of portions, to which portions were added the following stains in concentration of 6–10 drops of 1% stain solution per 50 cc. of agar: acid fuchsin, phenol red, methyl red, cresol red, and neutral red. A portion without stain was retained as a control. Samples of each portion were then poured into a number of sterile Petri dishes and inoculated with the three lilac strains of *Phytoph-*

thora. As certain of the stains used are pH indicators (phenol red, cresol red), the variations in pH during growth could be read directly. The original pH of the agar was 6.4. After 14 days *P. cactorum* and *Type A* had raised this to 7.4 in the areas occupied by mycelium, although *P. Syringae* had not changed the original pH of the agar. Methyl red appeared to have a repressing or toxic effect on *P. cactorum* and on *Type A*, but not on *P. Syringae*. On the other hand, neutral red exerted such an effect upon *P. Syringae* but not on the other two strains. None of the other stains appeared to affect the development of the mycelium or oospores.

All of the dyes used stained the oospore walls of *P. cactorum* and *Type A* brilliantly. (No oospores were formed in *P. Syringae*.) These were the only structures stained *in vivo* with phenol red, cresol red, methyl red, or acid fuchsin. In order to be certain of the presence or absence of vital staining, the mycelium was permitted to grow out on sterile cover-glasses which were later removed and examined for the presence of dye within the hyphae. Since *Phytophthora* is coenocytic, a dye which could penetrate should be carried out into such isolated hyphal stretches and thus be easily identified. The streaming of the protoplasm meanwhile offered a check as to the living condition of the hyphae. Neutral red penetrated the cytoplasm of the fungi and accumulated in the vacuoles, and could be identified far out on the sterile cover-glasses. This staining *in vivo* was accentuated when the mycelium was placed in a mildly alkaline buffer solution as an adjunct to staining. The staining of the vacuoles by neutral red but not by methyl red demonstrates that the vacuoles in these fungi are of the type designated by Bailey as "Type B" (3).

A marked reduction of the dye occurred in all the cultures containing methyl red, although such a dye reduction was not observed in the cases of the other dyes.

The evidence from this latter experiment accordingly confirms that obtained in the preceding experiments in demonstrating the similarity of reaction of *P. cactorum* and *Type A* in contrast to the marked divergence of *P. Syringae*.

B. MORPHOLOGY

Passing from a physiological to a morphological comparison of the three Phytophthora strains from Lilac, one finds that although there are certain resemblances there are also clear-cut and constant differences which readily distinguish the three strains from one another. The morphological comparison can best be pursued by considering successively the vegetative body, the asexual reproduc-

tive structures, the sexual reproductive apparatus, and finally the abortive reproductive structures so commonly found in this group of fungi.

VEGETATIVE BODY. The mycelium of all three strains of *Phytophthora* from Lilac is typical for the genus. In all cases the hyphae are highly granular and vacuolate, irregular in diameter and branching, and for the most part non-septate except at the bases of the reproductive structures and in old hyphae. Streaming of the protoplasm is customarily observed in all the strains, particularly in actively growing cultures in the main hyphae supplying the peripheral branch systems. This streaming is rapid and in all cases takes the form of an oscillation rather than a cyclosis as in the higher plants, the period of oscillation being from one to several minutes.

In order to obtain an accurate basis for comparison of the mycelial characters of the three strains, cultures were made on corn-meal agar and examined critically after one week's growth at room temperature. The following descriptions were made from examinations of such cultures.

In *Phytophthora Syringae* the branching is monopodial with the lateral branches irregularly distributed along the main axes and typically at right angles to the latter. The laterals are frequently constricted at the point of junction with the main axes and are poorly developed with relation to the main axes. Septations are very infrequent, are curved with the direction of curvature bearing no relation to the direction of growth, and are perforate. The diameter of the hyphae is variable, usually, however, fluctuating between 3 and 7 microns. The hyphae are granular or with extended vacuoles. Streaming is frequent and rapid.

In *P. cactorum* the branching is irregularly monopodial although sometimes approaching dichotomy. The lateral branches are more highly developed than in *P. Syringae*, are typically at right angles to the main axes, and are frequently much constricted at the junctions with the main axes. The hyphae are very granular and with fewer vacuoles than in *P. Syringae*. Septations are very infrequent, being found for the most part in empty hyphae, and are curved. The diameter of the hyphae is much more variable than in *P. Syringae* although the customary limits are between 3 and 7 microns. Streaming is frequent and rapid.

In *Type A* the hyphae are much more highly branched than in either of the preceding species on this substrate. The branching is typically monopodial but often becomes nearly dichotomous. The laterals may be perpendicular to the main axes, but more frequently they leave at an angle of between 50° and 70°. The hyphae, which

are finely granular, are more delicate and more even in diameter than in *P. cactorum*, varying between 2 and 6 microns in diameter. Septations are very infrequent and as in the species preceding are curved. The lateral branches are frequently constricted at their junctions with the main axes. Streaming is frequent and rapid.

The descriptions above apply exclusively to the aërial and superficial mycelium, the submerged mycelium being frequently very abortive in appearance. In all three strains there is a tendency for the mycelium of old cultures to assume an atypical condition, and this is particularly true of *P. Syringae*, where the hyphae of old cultures are often knotty, vesicular, and highly irregular.

In connection with the experiments in vital staining reported above, an interesting reaction to food supply was observed in *Type A* and later confirmed in the other species. Sterile cover glasses had been dropped onto the surface of the agar in newly made corn-meal agar cultures. The growing mycelium coming into contact with the sterile glass surface in many cases grew out onto the latter for a distance of one or more millimeters. As each hypha grew out onto the glass it produced a fairly complex system of lateral branches. Eventually, however, the dendritic system reached such proportions that the main hypha could no longer maintain a sufficient supply of nutrients for its needs. The reaction of the branch system was as follows. First a complete septation occurred near the edge of the cover slip in the main hypha. The protoplasm of the main hypha then retreated for a distance of about 10 microns and a second septation then separated the empty hyphal stretch from the retreating protoplasm. If any side branches lay along the unoccupied stretch of hypha, these also emptied into the retreating mass. Soon the process was repeated. A second retreat followed by a second septation was observed. This process continued until there were 10 or more septations in the empty section of hypha. Meanwhile the protoplasm of the more peripheral branches of the dendritic system was likweise receding, always marking the path of retreat by successive septations. In brief the protoplasm of the whole dendritic system was becoming condensed in the center of the dendritic mass, and the protoplasm was maintaining its life at the expense of a portion of itself. The remaining protoplasm was alive and active, and presumably would continue so until it had entirely consumed itself.

Asexual reproductive structures. The morphology of the asexual reproductive organs, in particular the zoosporangia, is of prime importance in the distinction of the strains of *Phytophthora* from Lilac, because herein lies the most important difference between *Type A* and *P. cactorum*, the two strains which up to the present have been seen to differ only in minor characteristics.

In general structure the sporangia of all three of the strains are wholly typical for the genus. They arise terminally on relatively undifferentiated sporangiophores, singly or in sympodial clusters, the successive sporangiophores always arising below the base of the preceding sporangium, never passing through the empty sporangium preceding as in the group of *P. cryptogaea*. No essential differences in development of the sporangia of the three lilac strains of *Phytophthora* have been observed. However, the character of the papilla of emergence of the zoospores is markedly different in the lilac Phytophthoras. The papilla of *P. cactorum* is of the more common form in the genus, prominent, sometimes even approaching cylindrical in shape. The papillae of the other two strains, on the contrary, are flattened, inconspicuous, crescentic in vertical section. That the type of papilla is a relatively fixed character is seen in the prominence given to this character in the recent taxonomic studies of the group. The papillae of *Type A* are so wholly distinct from those of *P. cactorum* that there is no danger of confusing the two even with a cursory examination of the sporangia, and in the many conditions of sporangium production observed, the character of papilla remained constant. That there is also a difference in the chemical composition of the papillae in *Type A* and *P. cactorum* is evident from the fact that the papillae of *P. cactorum* are often either dissolved or rendered invisible in Amann's lacto-phenol preparations, while those of *Type A* remain perfectly distinct under the same conditions. The types of papillae in the three lilac strains of *Phytophthora* are illustrated in Figures 1–24 of plate 46.

The lilac strain of *P. cactorum* is also distinguished from the other two strains by the fact that the sporangia are much more likely to be deciduous. If water cultures containing quantities of the sporangia of the three lilac strains of *Phytophthora* be shaken vigorously, many of the sporangia of *P. cactorum* will become detached, while those of the other two strains do not become detached to appreciable extent. The detached sporangia of *P. cactorum* each bear a tiny stump of the conidiophore, but these stumps are so short (being no more than half as large as the papillae of the same sporangia) that they could hardly be considered pedicels. In any case there is no evidence of the presence of pedicels in *P. Syringae* or in *Type A*, a point which will be considered in the systematic discussion to follow.

The mature sporangia typically emit zoospores in the customary fashion in the three lilac strains, and no significant differences in the three strains in mode of emission or in number, structure, or behavior of the zoospores were observed.

The released zoospores swim vigorously for a period of the order of an hour or less. They then come to rest, round off, lost their flagella, and germinate shortly. The rapidity of germination and the length of the germ tubes produced are astonishing. For example in one experiment in which freshly liberated zoospores were permitted to germinate in sterile pond water, the total length of hypha resulting from typical spores after 24 hours was measured. Such measurements gave for *P. Syringae* 270 microns, 315 microns, 210 microns, 227 microns, 217 microns, etc., for *P. cactorum* 210 microns, 112 microns, 158 microns, 227 microns, 158 microns, etc., and for *Type A* 402 microns, 356 microns, 140 microns, 368 microns, 315 microns, etc. One frequently observes that in the germination of the zoospores the limited amount of protoplasm passes to the tip of the growing germ tube, so that a germinated spore would typically show an empty spore case produced out into a long germ tube, the proximal portion of which would be empty, and the distal growing portion rich in protoplasm.

Studies were made of the measurements of the sporangia, but since no significant difference was found in the three strains under consideration and since the measurements of the sporangia of any one strain vary within extremely wide limits, no attempt will be made to differentiate the three according to this character. In the majority of sporangia of all three types the length varies from 20 to 40 microns and the width from 15 to 30 microns. There is also no significant difference in the ratio of width to length, this constant averaging in all the strains between .65 and .80.

SEXUAL REPRODUCTIVE STRUCTURES. The most fundamental morphological character in the separation of species of *Phytophthora* has been the type of antheridium, whether amphigynous (surrounding the oogonial stalk) or paragynous (not surrounding the oogonial stalk). Although Lafferty and Pethybridge (19) and others subsequently have shown that both amphigynous and paragynous antheridia may occur within the same species, yet all described species in which the sexual structures occur are characterized by having a distinct majority of the antheridia of one type or the other. The amphigynous type is by far the commoner in the genus, and it is very interesting to observe that the antheridia are chiefly paragynous in all of the lilac strains, although according to Tucker's conception (32) no other valid species than *P. cactorum* and *P. Syringae* possess a majority of paragynous antheridia. The antheridia of *Type A* are perfectly typical for the paragynous type and differ in no essential from those of *P. cactorum* and *P. Syringae*, as will be seen from an examination of Figures 27–30 of Plate 47. In all the

species here involved they are long persistent and are chiefly basal, only occasionally being lateral in position.

In all three strains the oogonia are broadly clavate to subspherical or spherical and usually terminal. The oospores are in all cases spherical, hyalin to light yellow, with the contents granular and variously vacuolate, and with a thick triple wall. The character of this wall appears to differ somewhat in *Type A* from the other two strains. In the latter it is smooth, while in *Type A* under some conditions it appears to be surrounded by a granular aura. The size of the oospores was investigated by employing statistical methods, as the variability is so great within a species as to require such a procedure. Measurements were made of 400 living oospores of each of the three strains, the measurements were grouped into classes, the frequencies plotted, and the constants calculated. The results are given in Text figure 1.

The following constants were derived from the data obtained:

	P. CACTORUM	TYPE A	P. SYRINGAE
Mean (in microns)	22.94 ± .06	24.97 ± .09	31.10 ± .11
Median (in microns)	22.79	24.92	31.68
Mode (in microns)	21.36	24.92	32.04

It will be seen that the measurements for *P. Syringae* are slightly greater than those found by other investigators (the mean usually being in the neighborhood of 28 microns). However, the difference between the measurements of *P. Syringae* and those of the other two strains is sufficiently great that it is considered significant in the distinction of the species. On the other hand, the difference in measurement between *Type A* and *P. cactorum* is so slight, in comparison with the variations within *P. cactorum*, that that difference is not felt to be significant for the purposes of species distinction.

Phytophthora omnivora is known to be heterothallic (22). Other species, such as *P. Cinnamomi*, *P. cryptogaea*, *P. Richardiae*, and *P. Phaseoli*, appear to be definitely homothallic (22). It was of interest, therefore, to look for any evidence as to the condition of sexuality in the lilac strains of *Phytophthora*. Such evidence was easily forthcoming, as an examination showed that in all three strains it was possible definitely to trace the origin of oogonia and antheridia from the same hyphal thread. Such a situation is figured for *Type A* and *P. cactorum* in Figures 29 and 32, 34 respectively, and has been illustrated by Klebahn for *P. Syringae* (18 p. 43, figs, 32, 33). Hence it may be maintained that all three of the strains in question are homothallic, at least customarily.

ABORTIVE REPRODUCTIVE STRUCTURES. Other types of reproductive structures than those considered above have been described

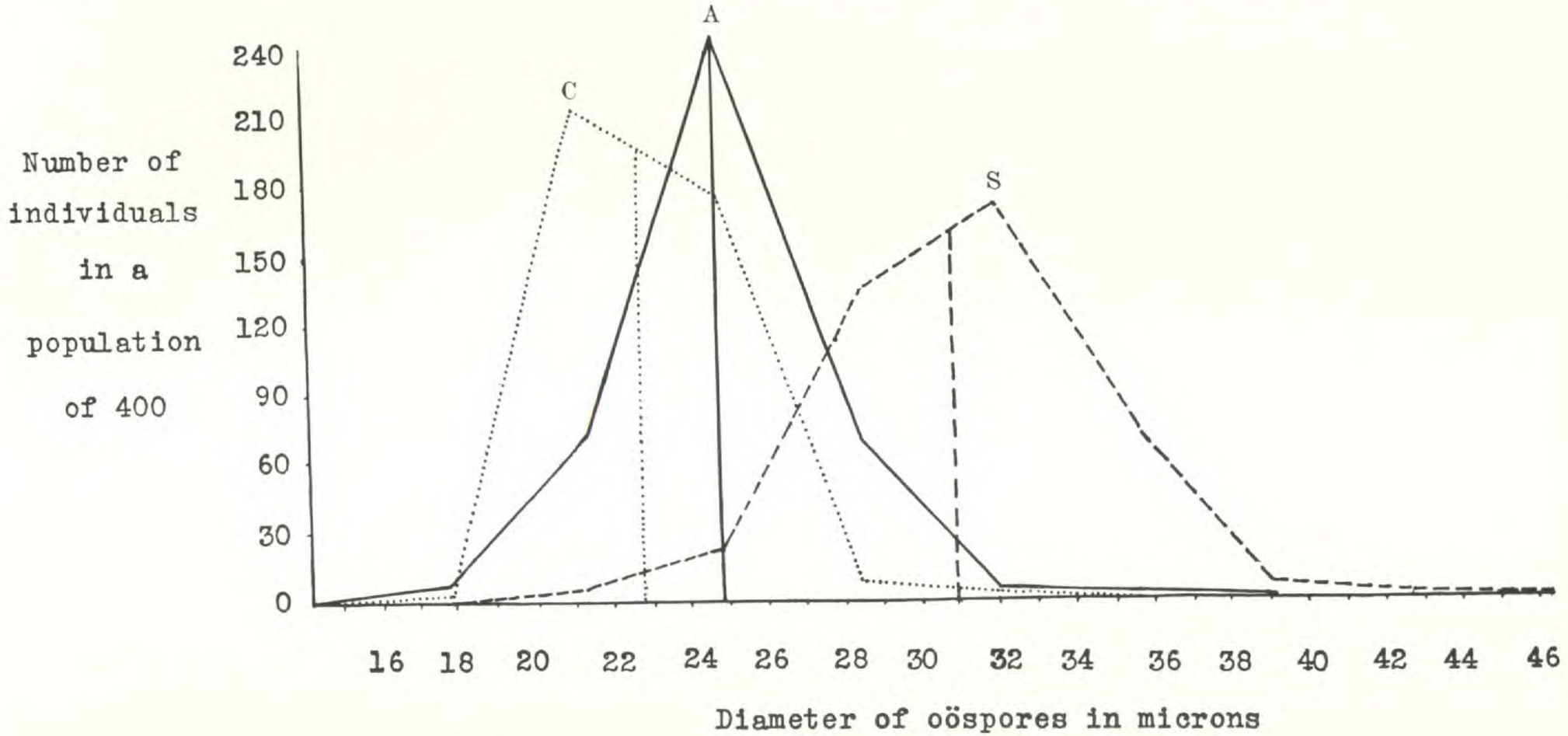

FIG. 1. OOSPORE SIZE IN THE LILAC STRAINS OF PHYTOPHTHORA

KEY: C. = P. cactorum; A = P. cactorum applanata; S = P. Syringae. The vertical lines represent the positions of the mean diameters.

in *Phytophthora*, as chlamydospores, pseudo-oospores, etc. There appears to be a certain amount of confusion as to the interpretation of these structures. In the strains of *Phytophthora* from Lilac such abortive reproductive structures have frequently been observed. Thus if one applies the Klebs technique to the fungi one finds that before the production of the great majority of sporangia, tentative attempts at sporangium-production end in an abortive fashion. The sporangia swell as in the typical fashion, but instead of maturing as sporangia a hypha continues out of the papilla region, and the sporangium becomes merely a more or less spherical dark swelling on the hypha. Frequently this is repeated several times in succession so that a chain of such swellings come to lie along the hypha. Although these might at first glance be interpreted as chlamydospores, the intermediate stages indicate that they are merely abortive sporangia formed when the conditions for sporangium-production are approached but not quite realized. This phenomenon is particularly noticeable in *P. Syringae*. At other times, particularly in old water cultures of *P. cactorum*, one frequently finds many "oospores" without antheridia. Whether these represent true oospores which have as yet failed to become fertilized, or whether they represent an abortive type of oospore or sporangium cannot readily be answered. Both the "unfertilized oospores" and the abortive sporangia, in any case, seem to be so dependent upon environment for their production and so erratic in occurrence that they are merely mentioned in passing, and no attempt will be made to use them in species distinctions.

C. SYSTEMATIC CONSIDERATIONS

With the background of comparative physiology and morphology as treated above it is now possible to orient the three lilac strains of *Phytophthora* within the genus. Since its original description *P. Syringae* has been felt to represent a well characterized and distinct species. Gäumann (12) considers it a race of *P. omnivora* de Bary, but the form of the sporangia together with other characters easily distinguish it from the *omnivora* group. The cultures of *P. Syringae* studied and reported in this paper conform in every respect to the original and to subsequent descriptions of *P. Syringae.*

Phytophthora cactorum (L. & C.) Schroet. includes a number of other species names according to the conceptions of various workers. Tucker's view of *P. cactorum* (32) includes *P. Fagi* Hartig, *P. omnivora* de Bary, *P. Pini* Leon., and its variety *Antirrhini* Sun. & Ram., and *P. Paeoniae* Coop. & Porter. Various species have been included within the concept of *P. cactorum* by Leonian and others.

No attempt will be made here to determine the species limits of *P. cactorum* in respect to the various allied species of the *cactorum-omnivora* group. Suffice it to say that all of the species so included are characterized by having the prominent type of papilla illustrated in this paper in Figures 9–16. Certainly *P. cactorum* is well distinguished from *P. Syringae*, although both typically have paragynous antheridia, by its prominent papillae and its very different power of growth and reproduction, even at temperatures at which *P. Syringae* utterly fails to make progress. The study of the cultures of *P. cactorum* considered in this paper shows the fungus to be perfectly typical to the earlier descriptions.

Passing then to the third lilac strain, *Type A*, one is confronted with the problem of ascertaining its systematic position. In the type of antheridia and in the majority of its physiological reactions it is manifestly similar to *P. cactorum*. The question then arises as to whether any description has been published of a *Phytophthora* bearing such a combination of characters as those described for *Type A*.

According to the treatment of Tucker (l. c.), the most recent thorough taxonomic treatment of the genus, *Type A* would necessarily fall within the group comprising *P. cactorum* and *P. Syringae* (with widely spreading growth on all ordinary media after 6 days at 20° C., and with predominantly paragynous antheridia). A number of species have been added to the literature since the publication of Tucker's work and these are here briefly considered.

In *Phytophthora gonapodiodes* (Peters.) Buis. (9) the secondary sporangia proliferate *through* the preceding sporangia in a fashion wholly different from that of any other *Phytophthora* species. *P. Porri* Foister, recently described on the Leek (11), is commonly characterized by apapillate sporangia and paragynous antheridia but it differs from *Type A* in the much larger oospores (32–33 microns) and proportionally much smaller antheridia. The descriptions of four species and varieties recently studied by Sideris have been made available through the kindness of Dr. Sideris (30). None of these, however, are identical with *Type A* since in *P. manoana* Sideris on *Ricinus* the sporangia are stipitate and papillate and sexual organs are absent, in *P. symmetrica* Sideris on *Ficus* the conidia usually germinate by a germ tube, and the sexual apparatus is absent, while in both *P. Meadii* var. *ananaphthora* Sideris and in *P. melongena* var. *ananaphthora* Sideris on *Ananas* the sporangia are prominently papillate and the antheridia predominantly amphigynous. A group of species from Formosa have recently been described by Sawada. Neither of the two species not included in

Tucker's treatment could be identical with *Type A* since in *P. Cyperi-rotundati* Sawada (28) the papillae are flat but the sporangia are pedicillate and the oospores are too large, averaging 30.8 microns, while in *P. Lepironiae* Sawada (= *Nozemia Lepironiae* [Saw.] Saw.) (27) the sporangia are entirely apapillate and the oospores very large, averaging 30–38 microns. *Phytophthora megasperma* Drechs. on *Althaea*, described by Drechsler in 1931 (10a), differs from Type A in the internal proliferation of the sporangiophores and in the very large size of the oospores (averaging 41.4 microns). Finally a new strain of *Phytophthora Syringae* has been very recently described by Ogilvie as causing a fruit rot of apples and pears in England and South Wales. This new strain, however, in morphology and in physiological reactions is plainly distinct from *Type A*. Thus Ogilvie records that the growth of this strain is poor or imperceptible on malt agar, bean agar, and corn agar, and that oospores are formed only on Quaker Oat agar, while it has already been pointed out that *Type A* vegetates and forms oospores profusely on these media. Ogilvie's strain also shows on potato-dextrose agar the knotty development of the mycelium so characteristic of *P. Syringae* and only rarely seen in *Type A*.

It is thus seen that *Phytophthora Type A* from Lilac differs strikingly from any described species of *Phytophthora*, while it resembles more closely *P. cactorum* than any other described species. It differs from *P. cactorum* in several respects, chief of which is the flattened nature of the sporangial papilla. It is therefore felt that *Type A* represents if not a distinct species at least a distinct variety of *P. cactorum*, and accordingly it is here so indicated. Since the structure of the papilla is the most striking differential character in comparison with *P. cactorum*, the name *Phytophthora cactorum* (L. & C.) Schroet. var. *applanata* n. var. is proposed. The diagnosis of this variety is here introduced.

Phytophthora cactorum (L. & C.) Schroet. var. **applanata,** n. var.

A typo differt sporangii papillis applanatis non prominentibus.

This variety differs from the species in the consistent presence of flattened, non-prominent sporangial papillae. Parasitic on the young growth of various horticultural varieties of *Syringa vulgaris* causing a soft dark decay. Hab. in Massachusetts, U. S. A.

V. COMPARATIVE PATHOLOGY OF THE SPECIES OF PHYTOPHTHORA PARASITIZING LILAC

Having inquired into the structure and behavior of the lilac strains of *Phytophthora* and established their identity, it now becomes necessary to consider more in detail the diseases caused by

them. The present section, accordingly, will deal with the nature of the diseases caused, reserving for the following section a treatment of the subjects of prognosis and control. The nature of the diseases of Lilac caused by *Phytophthora* species has been ascertained both by observations of the symptoms and of predisposing factors to the diseases as they occur in the field and also by infection experiments. These sources of evidence will accordingly be treated in turn. It must be stated at the outset that the disease caused by *P. Syringae* does not appear to occur in America, and accordingly the description of this disease is based upon the fairly extensive published accounts.

A. SYMPTOMS

According to Klebahn (18) the disease caused by *Phytophthora Syringae* manifests itself first by an inhibition of development of the buds of the younger shoots. The cortex of the diseased twigs is killed with the result that the bark of the diseased twigs is darker than that of healthy twigs and somewhat shrunken. Usually such killing of the cortex involves the whole circumference of the twigs. The vascular system is not affected in the earlier stages of the disease but eventually it becomes interfered with and the whole of the affected twig dies. Occasionally the disease may be limited to a single bud or part of a bud. Such symptoms become noticeable only at the time of spring development, although the fungus may be actively killing the cortex during the winter.

Miss de Bruyn has pointed out (6) that *Phytophthora Syringae* may also attack the leaves, causing irregular brown patches with lighter margins. If the *Ovularia Syringae* of Berkeley is the same fungus as *Phytophthora Syringae* (Kleb.) Kleb., as is probable, then Berkeley's description of the brown leaf infections of lilac (4) harmonizes with the findings of Miss de Bruyn. Arnaud has also observed the same type of leaf infections (1, 2). Oospoores are customarily found in the infected tissues, and in moist weather zoosporangia are also produced, the latter emerging through the stomata as in the case of *Phytophthora infestans* according to Berkeley. Finally the infection experiments carried out in connection with the study reported in the present paper indicate that *Phytophthora Syringae* is also able, under some conditions, to attack the succulent young tissues in the same fashion as is characteristic of the other two lilac strains of *Phytophthora*.

According to White's description (35) the infection caused by *Phytophthora cactorum* takes place in the succulent young shoots. "A die-back condition is caused by the invasion of the fungus in the cortex of the shoot, and in some cases suckers three and four feet

long have been killed to the ground. Frequently the entire crop of root suckers which arise about the base of old lilac bushes have been completely killed. On leaves, infections first appear as small, water-soaked areas, increasing in size during periods of rain, but drying out during periods of dry weather. Entire leaves may be invaded, and the fungus has been found growing down the petioles into the cortex of the branch where dark brown or black cankers are produced."

The disease observed in Boston follows in general the same course as that in New Jersey. Infections of the young shoots are observed soon after the buds have started development, and the disease is maintained through the summer months in the supply of soft young suckers which are constantly being formed at the bases of older lilac plants. Hardening of the wood completely checks the disease provided it has not advanced too far for the recovery of the shoot. The stem lesions appear to be more frequent, somewhat resembling those caused by *Bacterium Syringae* in being elongated, dark, and soft, but becoming hard and calloused in the cases where the shoots recover. Oospores are frequent in the diseased tissues, and sporangia are produced on the surfaces of the lesions during moist weather or if the lesions are placed in moist chambers.

The disease caused by *Phytophthora cactorum* var. *applanata* in all essential features resembles that caused by *P. cactorum*, so much so that no additional description is necessary, the lesions being confined to the succulent tissues, being dark, soft, and elongate, and becoming checked by the hardening of the tissues affected.

B. PREDISPOSING FACTORS

Phytophthora Syringae, as may be seen from the work on temperature relations of the fungi, is an organism favored by cool weather. It was noted above that its growth ceases at 25° C. (77° F.), and as this temperature is frequently exceeded in the spring and summer months it is not surprising to find that the disease caused by *P. Syringae* is essentially a disease of winter and early spring. Klebahn (18) found the disease to be primarily one of plants which had been closely packed in cool chambers for the winter and covered with leaves preparatory to forcing in the spring. Miss de Bruyn (6), in investigating more accurately the relation between season of the year and occurrence of the disease, found that a maximum number of successful stem inoculations could be made in November, the number decreasing to practically zero in the summer, and again increasing in the fall. The same was true of bud inoculations which reached a percentage of successful inoculations of 100% in Novem-

ber and December and fell to 0% in September. Moreover, she also reported that the increase in length of stem inoculations was greatest in February, falling to zero in July and again beginning a gradual increase in November.

On the contrary the other two Phytophthora strains causing lilac disease are fungi favored by higher temperatures. Accordingly it is consistent that the lesions caused by the latter are more prevalent during the spring and summer months and are absent during the winter.

Moisture plays an important role in the severity of all of the Phytophthora diseases of Lilac. Spread of the diseases in all cases is chiefly through the activity of the zoospores, the oospores being non-motile and buried within the tissues, to be freed only by the disintegration of the diseased parts. A high degree of humidity is essential to the production of sporangia, and water is necessary for the life and distribution of the zoospores. Therefore the rate of spread of the diseases is conditioned by the rainfall and humidity. Herein lies one reason for the destructiveness of the disease caused by *Phytophthora Syringae* in the European forcing beds. The plants which had been stored were covered with a moisture retaining layer of leaves which greatly facilitated the dispersal by zoospores. The increase in size of *P. cactorum* lesions in periods of rain has been noted above, and White has also observed that excessive shade and relatively high humidity are conducive to the spread of the disease caused by *P. cactorum*. Excessive rainfall also exerts a secondary influence upon the severity of the diseases caused by *P. cactorum* and its variety *applanata*. It is a matter of frequent observation that prolonged rainy seasons prolong the period before the new growth begins to harden. Accordingly, as the period of succulence is extended the opportunity for infection and enlargement of the lesions becomes proportionally increased. In the case of the disease caused by *P. Syringae*, rainfall has still another unfavorable influence. Miss de Bruyn has observed (6, 7) that during seasons with abnormal rainfall in August and September the development of the fungus concerned upon the leaves is greatly aided while leaf fall is retarded. The development of the fungus becomes sufficient, under such circumstances, that the fungus can pass down the petioles and into the cortex of the stems, thus establishing itself in the woody tissue and offering a source of infection for spread during the winter months.

As another predisposing factor should be mentioned the methods of planting and of cultivation. If Lilacs are so heeled in that the buds come to lie in proximity to the soil they are in a favorable

situation for infection, since *Phytophthora* species are able to exist saprophytically in the soil. Such a method of planting is frequently employed, and Klebahn called attention to the destructiveness of the infection by *P. Syringae* resulting. Lustner (24) observed that of two similar groups of Lilacs, one of which had been planted in normal fashion, the other heeled in, only the latter group suffered from the disease caused by *P. Syringae*. In the case of the diseases due to *P. cactorum* and *P. cactorum applanata* the method of cultivation may likewise exert an influence upon the severity of the disease. As it has been shown above that these diseases are maintained during the summer upon the succulent young suckers at the bases of old lilac plants, it is manifest that clean cultivation involving the continual removal of such suckers will decrease by much the amount of inoculum available the following spring. Such suckers improve neither the appearance nor the health of mature specimen Lilacs, and clean cultivation is hence doubly desirable.

A fourth predisposing factor may be injury. Klebahn was unsuccessful in causing infection with *P. Syringae* to woody stems unless these had been previously injured, and accordingly it is probable that infection in nature is facilitated by injuries. However, in the cases of the diseases caused by the other two lilac strains of *Phytophthora*, infection of the succulent growth appears to be independent of injury.

C. INFECTION EXPERIMENTS

In 1906 Klebahn succeeded in transmitting the disease caused by *P. Syringae* from Lilac to Lilac by means of pieces of infected cortex inserted into healthy lilac stems (17). In 1909 he reported a more extensive series of infection experiments using pure cultures of the fungus (18). He was able to secure satisfactory infections in wounds in the woody stems as well as in uninjured buds using mycelium as the inoculum, and likewise to infect successfully using pure cultures of swarm spores. He also carried out a series of infection experiments demonstrating that the fungus was not only able to infect Lilac but that it would likewise infect a variety of other related and unrelated hosts such as *Jasminum*, *Forsythia*, *Crataegus*, *Pyrus*, *Prunus*, *Acer*, *Aesculus*, *Alnus*, *Corylus*, *Quercus*, and *Tilia*. Negative results were obtained with a number of other inoculated subjects.

De Bruyn's inoculation experiments with the same fungus (6) have already been mentioned. It will be recalled that she was able to obtain a high percentage of infections on lilac during the winter, using mycelium as inoculum. The only other infection experiments with *Phytophthora* from Lilac are those of White in 1929 with *P.*

cactorum. White mentions (35) that he has successfully performed repeated cross inoculations with this organism between Lilac and Rhododendron, which latter is also parasitized by *P. cactorum* in nature.

During 1930 and 1931 a number of infection experiments were performed by the writer with all three strains of *Phytophthora* from Lilac. These will be summarized at this point.

On April 2, 1930 ten pot plants of *Syringa vulgaris* var. *purpurea* with stems of 1 cm. diameter were inoculated in knife wounds of the woody stems with mycelium from agar cultures of *Phytophthora Syringae*. The inoculations were bound with wet sphagnum and raffia in the conventional manner and placed in a large moist chamber. On examination five months later all the inoculations were negative. The failure was probably due to the time of year of inoculation since Miss de Bruyn likewise obtained almost negative results in inoculations performed in April.

The same experiment was repeated the following December using 24 similar host subjects and inoculating eight each with *P. Syringae*, *P. cactorum*, and *P. cactorum* var. *applanata*, respectively. The inoculations were performed as in the preceding experiment except that in some of the plants semi-woody tissues were inoculated, while in other plants only the woody stems were employed. The extent of spread of the lesions was observed three months later. The following table gives the results.

AVERAGE INCREASE IN SIZE OF LESIONS (mm.)

INOCULATION IN	P. SYRINGAE	P. CACTORUM	P. CACTORUM APPLANATA
Hard wood.....	23.0	17.2	17.6
Semi-woody tissues.....	1.2	4.0	3.0

The respective fungi were reisolated from the margins of the majority of the lesions three months after inoculation. The results indicate that although *P. Syringae* is most virulent in woody tissues, *P. cactorum* and *P. cactorum applanata* can also cause infection in such tissues. Since the inoculated plants were in a greenhouse the temperature of which was moderately high, *P. cactorum* and *P. cactorum applanata* were growing at a temperature favorable for their development and one which would not obtain at this season in nature.

A third infection experiment was performed in April, 1931, differing from the preceding in that the normal succulent growth was selected as the infection court. The inoculum was in the form of pure cultures of the three lilac strains of *Phytophthora* on agar. In some subjects a pair of leaves was removed from each succulent

shoot and the inoculum bound over the leaf scars, in others no injury was resorted to. The inoculations were then surrounded with moist cotton and raffia and placed in moist chambers. Five plants each were used for each strain of fungus. The results were as follows:

HOST INOCULATED	ORGAN INOCULATED	P. SYRINGAE		P. CACTORUM		P. CACTORUM APPLANATA	
		INOCUL'S	INFECT'S	INOCUL'S	INFECT'S	INOCUL'S	INFECT'S
Syringa vulgaris	Succulent shoots	14	(2) (Very weak)	30	28	15	6
Syringa vulgaris	Blossoms					1	1
Ligustrum ovalifolium	Succulent shoots			12	2	8	(1) (Very weak)
Ligustrum ibota	Succulent shoots			2	0	2	0

In a number of instances in the infections with *P. cactorum* and *P. cactorum applanata* there was clear-cut evidence from the limits of the infections produced that the respective fungi had succeeded in penetrating through the uninjured epidermis. This experiment indicates that under more nearly natural conditions in the spring *P. cactorum* is highly virulent to succulent lilac tissues, *P. cactorum* var. *applanata* somewhat less so, and *P. Syringae* very weakly aggressive.

In the typical negative inoculations with *P. Syringae* indicated in the table there was frequently a small brown area marking the original entry of the fungus, but around this developed a layer of callus tissue, cutting off the lesion from the rest of the stem tissue. With the employment of *P. cactorum* and *P. cactorum* var. *applanata* the typical lesion took the form of a brown, soft area which appeared within 48 hours of the time of inoculation. Such brown areas were not necessarily restricted to injured points on the shoot. From the brown area watery streaks extended up and down the stem, looking as though the cells of the streaks had been mechanically crushed. Very rapidly these streaks turned brown and soft until the advance involved several centimeters after 96 hours. The shoots would then fall over at the rotted part and the distal portions would die. Cut leaf bases were readily occupied by both the fungi, but there was a partial checking of fungus growth at the layer of abscission, that is, the fungus appeared to pass that layer with some difficulty. The lesions were usually covered with *Phytophthora* mycelium when removed from the moist chamber.

The evidence from the preceding experiment shows that under some conditions *P. Syringae* is able weakly to infect succulent lilac tissues. In order to test this matter further, however, an experiment was set up in April 1931, using zoospores as inoculum. Glass

collars were fitted around the stems of 15 pot lilacs (*Syringa vulgaris purpurea*) each with a succulent shoot at the top of the stem and partly within the glass collar. The glass collars were fashioned and sealed in the manner described by Klebahn (18). Into the collars of ten of the plants were then poured pure cultures of freshly liberated zoospores of *P. Syringae*. The remaining 5 plants were similarly treated but using sterile water instead of the zoospore suspensions. The following day a second dose was given to 8 of the experimental plants and to four of the controls. The day after a third dose was given to 6 of the 8 mentioned above and to 3 of the controls. This process was repeated until the last two of the experimental plants had received 5 daily doses of the zoospore culture and the last control 5 doses of sterile water. The plants were later examined for infection. The results of the examination are given in the following table:

Number of days of treatment	Treated with Pure culture of zoospores of P. Syringae	Sterile water (Control)
1	Slight infection	No injury
2	Typical infection	Slight browning
3	Typical infection	No injury
4	Typical infection	No injury
5	Typical infection	Slight browning

This experiment therefore, indicates that although *P. Syringae* is primarily a fungus causing disease in woody tissues of Lilac and in the winter, nevertheless, it is able under some conditions to cause an infection of succulent tissues in a manner similar to *P. cactorum* and *P. cactorum applanata.*

Summarizing the results of all the infection experiments performed to date with the lilac Phytophthoras, one can say that *P. Syringae* has been shown to be capable of causing infection in injured woody stems during the winter months but not during the summer months, in uninjured buds during the winter months, and in more succulent tissues under certain conditions in the spring and summer. However, it is primarily a parasite of dormant tissues. *P. cactorum* and *P. cactorum* var. *applanata*, on the contrary, have been proved to be more readily capable of causing disease in succulent tissues during the spring and summer than *P. Syringae.* On the other hand, although these latter fungi are also capable of parasitizing dormant tissues, they are less aggressive in such tissues than *P. Syringae.* The inoculation experiments thus confirm the observations that in nature *P. Syringae* is primarily the cause of a disease of dormant Lilaçs, *P. cactorum* and its variety *applanata* primarily the causes of diseases of succulent young sprouts.

VI. PROGNOSIS AND CONTROL

The final chapter of such a study as the present one, and from the practical standpoint the most important one, necessarily must be concerned with the control of the diseases in question. However, before proceeding directly to the question of control it is advisable to consider briefly the conditions under which the diseases are likely to be most severe and to warrant active steps in control.

The Phytophthora diseases of Lilac under ordinary conditions are not as destructive as are many of the more devastating diseases of other economic plants. There is little likelihood of their increasing to epidemic proportions, at least under American conditions of lilac culture. On the other hand, given optima of moisture, temperature, and supply of inoculum, the diseases may well merit serious measures for control. This fact is particularly true of an ornamental plant such as the Lilac where infections of a severity which would not seriously diminish the quantity of product in crop plants, may in ornamental plants, nevertheless, diminish very appreciably the market or aesthetic value of the host.

The disease caused by *P. Syringae* in the European lilac forcing industry as well as in nurseries and private collections has already shown itself capable of warranting prophylactic measures. This has been particularly true in seasons in which there has been an abundance of rain in August and September and under cultural methods which facilitate the development of the lesions and the spread of inoculum. Such methods are those by which Lilacs are closely packed in relatively air-tight chambers, preparatory to shipment, transplantation, grafting, or forcing. On the other hand, where Lilacs are well spaced, growing under more or less natural conditions outdoors in the private collection or nursery, the spread of the disease is much less readily accomplished, and control by natural agencies will probably be relatively effective.

As regards the other diseases caused by *Phytophthora cactorum* and *P. cactorum* var. *applanata*, the conditions favoring the diseases are somwehat different. Here the diseases are more likely to become epidemic in natural plantings, particularly during those seasons in which the spring rains are prolonged. Moreover, as spring is the season of the year during which the Lilac attracts most attention, infections in the foliage and blossoms in the spring are doubly important. Hence it will be seen that the latter diseases may at times be rather important under American conditions of lilac culture. It certainly will be to the advantage of the lilac grower to examine his plants during early spring and up to blossoming time for Phytophthora infections, especially during the more rainy seasons, and to

take prompt steps to check any serious impending spread of infection.

The methods of prophylaxis against a Phytophthora disease in Lilac depend upon the species of *Phytophthora* involved. Control of the European disease, caused by *P. Syringae*, has been worked at rather at length by both Klebahn and de Bruyn. Klebahn's suggestions (18) include the removal and burning of all infected plant parts, cultural methods which eliminate the close heeling-in of stored Lilacs, maintainance of a low degree of humidity in stored lilacs especially by elimination of leaves as a covering for such plants, and finally avoidance of mechanical injuries to the Lilacs by careful cultural practices. After a few years of observance of such suggestions, Klebahn felt that the disease had been somewhat diminished in frequency. He suggested that fungicides, used during storing and eventually in the nursery, might be of value, but he did not test their efficacy. Miss de Bruyn (6) originally added to Klebahn's suggestions the desirability of removing the infected leaves by hand during such years as show an abnormal rainfall in August and September, but a later study (8) showed that the quality of the blooms was so decreased by such treatment that the method was not considered advisable. She also suggested Bordeaux and lime-sulphur sprays, cautioning at the same time against spray injury to the young tissues.

As regards the other Phytophthora diseases of Lilac, the experimental conditions have not been favorable to a thorough comparison of control measures. However, a knowledge of the success of prophylactic treatments in diseases caused by similar fungi and of the physiology of the fungi involved here leads one to a number of *a priori* suggestions. White's recommendations for the control of the disease caused by *P. cactorum* (35) embody avoidance of planting of Lilacs near to Rhododendrons (since the fungus involved also parasitizes Rhododendron), removal and burning of dead wood, soil sterilization where practicable, discreet pruning, and spraying with a dormant application of lime-sulphur together with summer applications of Bordeaux.

In the control of both of the latter diseases good cultural methods should prove most useful. The ideal Lilac, according to most growers, is a single-stemmed plant of the general form of an inverted cone. An abundant crop of suckers at the base detracts both from the aesthetic value of a plant and from its ability to blossom. Moreover, it is the suckers which materially aid in carrying the infection through the summer. Hence the complete and regular removal of such suckers is to be advocated. Combined with this, care should

be taken to keep the bushes properly spaced and thinned out in order to avoid excess of moisture and shade. During the spring, particularly during wet seasons, frequent examinations should be made for Phytophthora lesions on the young growth, and if such lesions are found they should be immediately destroyed. If lesions occur to a considerable extent on the shrubs, protective applications of Bordeaux are indicated, and without delay, since the spread of the diseases is very rapid under such conditions. It has been suggested that the spotting caused by the Bordeaux may be deleterious to the appearance of the plants. Such an objection would not apply to the nursery, but in private collections the spotting might be avoided by the substitution of one of the non-spotting fungicides now on the market. Methods of soil sterilization for the most part could not be conveniently applied to standing lilacs, but under the conditions of some propagation practices it would be well to consider soil sterilization in case of a severe attack by *Phytophthora cactorum* or *P. cactorum applanata* on nursery stock.

The efficacy of Bordeaux spray and sulphur dust as protective agents were investigated in the following manner. Water cultures of the three Phytophthora strains were prepared in order to obtain suspensions of freshly liberated zoospores. Drops of the zoospore suspensions of each fungus were then placed on microscope slides which had been in one case dusted with a reputable commercial sulphur dust, in a second case sprayed with a 4-5-50 Bordeaux mixture, and in the third case untreated. Examinations were first made for loss of motility of the zoospores. It was found that with *P. Syringae* the untreated spores remained active for about an hour, the Bordeaux treated spores lost all motion within 5–10 minutes, and the sulphur treated spores lost all motion in about 20 minutes. With the spores of *P. cactorum* the controls remained active for nearly an hour, and those treated with Bordeaux and sulphur for 10–20 minutes. With *P. cactorum applanata* the controls remained active for nearly an hour but all motion was lost in the spores treated with both Bordeaux and sulphur in less than 5 minutes.

As regards germination, the control spores of *P. Syringae* germinated to an extent of about 90% in 24 hours, those of *P. cactorum* to about 99%, and those of *P. cactorum applanata* to about 80%. Meanwhile the Bordeaux treated spores of all three strains completely failed to germinate, while the sulphur treated spores germinated in *P. Syringae* 85%, in *P. cactorum* 77%, and in *P. cactorum applanata* 70%.

This experiment accordingly indicates that Bordeaux of a strength of 4-5-50 will completely inhibit zoospore germination in all the

lilac strains of *Phytophthora*. Hence in the field one would anticipate that Bordeaux would offer a very satisfactory protection, since infection in the field is apparently almost or quite exclusively by zoospores. On the other hand, sulphur dusting would not be expected to give satisfactory results since it exerts very little inhibiting effect upon zoospore germination.

VII. SUMMARY

1. The present paper reports a comparative study of three strains of *Phytophthora* parasitizing Lilac, and of the diseases caused by them. The fungi involved are *Phytophthora Syringae* (Kleb.) Kleb., *P. cactorum* (L. & C.) Schroet., and a third strain here tentatively referred to as *Type A*.

2. The physiological behavior of the three strains of *Phytophthora* was investigated with the following results:

a. In regard to rate and type of growth upon a variety of artificial substrata, *Phytophthora Syringae* was found to differ markedly in its reactions from the other two strains, while the latter, although in general similar to each other, showed certain suggestive differences.

b. With respect to production of reproductive organs upon a variety of artificial media, *P. Syringae* again proved to be entirely distinct, while certain minor differences distinguished *P. cactorum* and *Type A*.

c. In relation to temperature, *P. cactorum* and *Type A* were found to vegetate luxuriantly at temperatures as high as 25° C, at which temperature *P. Syringae* failed to make any growth. The optimum temperature for the former two strains was found to be in the neighborhood of 25° C., in contrast to 20° C. for *P. Syringae*.

d. The strains manifested corresponding differences with regard to pH. *P. Syringae* vegetated well between pH 3.5 and 7.0, growth virtually ceasing at 7.5, *P. cactorum* showed good growth in the very long range from 3.0 to 10.0, while *Type A* grew satisfactorily between 3.0 and 9.5. In passing, the subject of vital staining of the lilac strains of *Phytophthora* was investigated. Staining of the vacuoles was observed with neutral red but not with methyl red, indicating that the vacuoles of these strains of *Phytophthora* are of the type designated by Bailey as Type B. Methyl red was not reduced by *P. Syringae* but was reduced by the other two strains. The oospore walls of *P. cactorum* and *Type A* were brilliantly stained *in vivo* by a number of the stains employed.

3. The morphology of the three strains of *Phytophthora* was likewise investigated with the following results.

a. Minor, but no important differences were observed in the mycelial characters of the three strains.

b. The sporangia differed markedly with respect to the type of papilla of zoospore emergence. That of *P. cactorum* was strikingly different from those of *P. Syringae* and *Type A* in being very prominent, in contrast to the flattened, inconspicuous papillae of the other strains. This character was striking and constant, and was the most useful criterion for distinguishing the otherwise similar *P. cactorum* and *Type A*.

c. Few significant differences were observed in the sexual apparatus of the three strains. The antheridia were mainly paragynous and the mycelia homothallic in all the strains. There was, however, a significant difference in oospore size between *P. Syringae* and the other two strains.

d. Abortive reproductive structures are discussed as they occur in the three strains.

4. On the basis of the work on the comparative physiology and morphology of the three strains, their respective systematic positions are discussed. *Type A* was found to resemble *P. cactorum* more closely than any other species of the genus. However its different form of papilla, together with other more minor differences, have lead to its recognition as a new variety of *P. cactorum*, namely *P. cactorum* var. *applanata*, n. var., the diagnosis of which is given.

5. The comparative pathology of the three lilac strains of *Phytophthora* was investigated. *P. Syringae* was found to differ markedly from the other strains in the type of lesion produced, the tissues attacked, and the time of year of greatest fungus activity. *P. cactorum* and *P. cactorum* var. *applanata* behaved in a similar fashion pathologically. The symptoms caused in Lilac by the three strains of *Phytophthora* are described, the factors predisposing to the diseases caused are analyzed, and the pathogenicity of the three strains under various conditions determined by infection experiments.

6. On the basis of the comparative study of the fungi and of the diseases caused by them, the probable severity of the diseases is discussed, together with the conditions under which the diseases are likely to prove most destructive. Finally the possible methods for the control of the diseases are considered and recommendations made as to the prophylactic practices found desirable.

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